THE EFFECTS OF THE IODIDE ION ON ABSCISSION IN BEAN LEAF EXPLANTS

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MABEL L. ALLEN

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ABSTRACT

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The Effects of the Iodide Ion on Abscission in Bean Leaf Explants

Advisor: Dr. Johnny Jackson

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This investigation was undertaken in order to study the effects of the iodide ion on the formation of the abscission zone in leaf explants of 23 to 27 day old bean plants.

The abscission zone was excised from the terminal leaflet of the first trifoliate leaf of the plants. The proximal and distal ends of the tissue were treated with 5 concentrations of potassium iodide (1.0, 2.0, 3.0, 4.0, 5.0 X 10^{-4} M), either incorporated into plain agar blocks, or agar blocks containing a promotive level of IAA (200 mg/l), or an inhibitory level of IAA (500 mg/l). The results showed that the iodide ion considerably retarded abscission in the bean explants. The two levels of IAA, and the different sites of application had no appreciable effect on the inhibitory action of KI.

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CHAPTER I

INTRODUCTION

Boyd (6) reported in the patent literature that water soluble inorganic iodide salts when applied to the leaves of crop bearing plants nearing maturity induced substantial defoliation. Later Nolan (16) reported similar results with the use of elemental iodine. While screening certain synthetic compounds for herbicidal activity, Herrett, Hatfield, Crosby, and Vlitos (10) noticed that various organic iodide complexes and simple iodides such as salts of alkali metals caused marked defoliation when applied to certain legumious plants. Since auxin appears to be the most important factor regulating the onset and rate of abscission, Herrett et al. (10) suggested that some intimate relationship apparently exists between the iodide induced abscission and the indoleacetic acid (IAA) level in the plants.

The role of auxin in leaf abscission has been recognized since the early 1930's (13). Yet, the exact mechanism through which auxins exert their effects is still not clear. Carns (7) stated that in recent, and separate reviews Addicott, Jacobs, Rubenstein, and Leopold assigned a different and specific regulatory role to auxin in leaf fall. However, they generally agreed that the onset and rate of abscission is brought about by a lowering of the auxin concentration. A defoliant generally is believed to exert its effect by interfering either directly or indirectly with the auxin level across the abscission zone. Prior to the above reports of Boyd (6), Nolan (16), and Herrett et al. (10), the only iodide

compound that seems to have been used in any appreciable degree as a defoliating agent has been 2, 3, 5,-tri-iodobenzoic acid (TIBA).

This investigation was undertaken in order to study the effects of the iodide ion on the formation of the abscission zone in leaf explants of bean plants.

CHAPTER II

REVIEW OF LITERATURE

Leaf fall with age is a common phenomenon among many plants. Generally it has been found to be associated with a separation layer or abscission zone which usually forms at the base of the abscissing organ. The exact role of this layer in leaf fall is not well-defined because certain deciduous plants shed their leaves without producing such a layer (14). In leaves where the abscission layer usually develops, its initiation may be brought about by several factors. Among them are low temperatures, application of growth regulators, a water deficit, removal of the leaf blade, reduced light, and the application of defoliants (14).

In 1936, LaRue (12) demonstrated that the abscission of debladed petioles in Coleus plants could virtually be completely inhibited by capping the stump with lanolin paste containing IAA. Since that time numerous reports substantiating his work, with Coleus and other plants, have appeared in the literature (1, 2, 3, 5, 8, 9, 11, 15). The work of Addicott and Lynch (2) showed that abscission could either be accelerated or retarded by IAA depending upon the locus of application and concentration. Relatively high auxin levels generally retard the formation of the abscission zone whereas lower concentrations have the reverse effect. Shoji, Addicott, and Sivets (17) studied abscission in the trifoliate leaf of Black Valentine beans and found that a moderately high auxin level, which was considerably above that of the stem, was maintained in the blade throughout its normal functioning. It was further noted that before

the leaflets abscissed, their auxin concentration declined to the level of that found in the stem. They concluded that it was the auxin gradient across the abscission zone that controlled leaf fall and not the actual amount. After a more extensive and probing investigation of abscission, Addicott, Lynch, and Carns (3) came to the same conclusion as Shoji et al. (17) as to the role of auxin in abscission.

The work of Gaur and Leopold (9) with explants of Red Kidney beans indicated that abscission was apparently controlled by the quantity of auxin and not by the auxin gradient as was proposed by Shoji et al. (17), and Addicott et al. (3). They stated that a low concentration of auxin at the abscission zone accelerated the process, whereas a high concentration inhibited abscission. Since Addicott et al. (3), and Gaur and Leopold (9) proposed two different theories regarding the effects of auxin on abscission, their work was reinvestigated by Biggs and Leopold (5) with the use of explants from Red Kidney beans. They concluded from their study that the primary action of auxins is directly on the abscission zone and is of a two-phase type with low concentrations accelerating abscission and high concentrations inhibiting abscission.

The exact mechanism through which auxins exert their effects on leaf fall is not clear. However, most studies show that factors which interfer with its action or cause a decline in its concentration usually have accelerating effects on abscission. Ethylene, a naturally occurring defoliant, has been found to promote abscission and also break auxin induced bud dormancy by causing, in some way, a drop in the auxin level (4). Synthetic defoliants, also, have been found to greatly reduce the auxin level of leaves. Audus (4) states that the more widely used synthetic

defoliants are highly toxic substances and suggest that their defoliating action is probably due more to leaf injury rather than to the direct action on the auxin.

The work of Herrett et al. (10) showed that the iodide ion could induce premature abscission in the leaves of Tendergreen beans. Their work which was the only extensive report found in the literature supported the statements of Boyd (6), and Nolan (16). Herrett and co-workers (10) noticed that abscission induced by the iodide ion was preceded by leaf blade desiccation and concluded that this reduced the auxin content which resulted in leaf fall.

CHAPTER III

MATERIALS AND METHODS

Bean leaf explants (Phaseolus vulgaris variety Tendergreen) were used as experimental plant material. The plants were grown from seeds planted in loam soil under normal greenhouse conditions for 23 to 27 days. The bean explants were excised from the terminal leaflet of the first trifoliate leaf so as to include 10 mm of rachis and 3 mm of pulvinus tissue (Fig. 1).

Potassium iodide obtained from the J. T. Baker Chemical Company served as the source of the iodide ion. Three different groups of experiments were performed. For Group A, 5 different concentrations of KI (1.0, 2.0, 3.0, 4.0, 5.0 X 10^{-4} M) were incorporated separately into 1.5% agar, and plain agar served as the control. After forming a gel the agar was cut into uniform blocks (13 mm^3).

For each iodide treatment the leaf explants were inserted separately into pairs of agar blocks that contained the desired concentration of potassium iodide. Both ends of the controls were placed into plain agar blocks. Five similarly treated tissue explants were placed horizontally into separate Petri plates, covered, and kept at room temperature on a laboratory table for 5 days (Fig. 2). Each plate was checked daily for abscission by pressing lightly on the pulvinus with a small glass rod. For the first test (T-1A) in Group A, the proximal ends of the explants were subjected to the 5 KI concentrations and the distal ends were placed in plain agar blocks. The proximal ends of the excised leaf tissue in

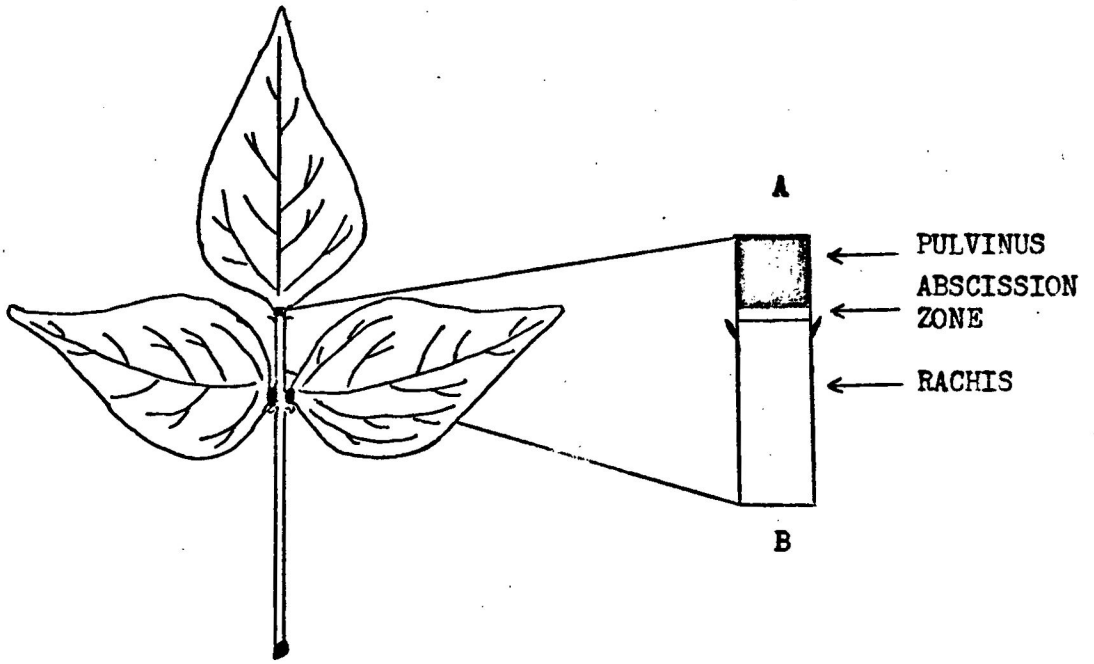

Fig. 1. Diagram of a trifoliate bean leaf showing the position of explanted tissue and with indications of the positions in which agar blocks were placed. A - distal, B - proximal.

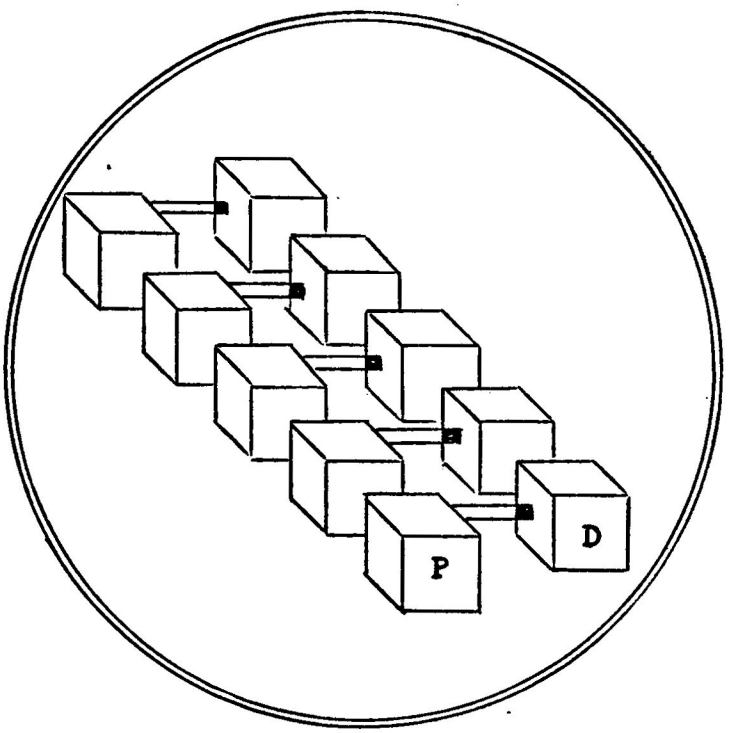

Fig. 2. Diagram showing how the ends of leaf explants were inserted into agar blocks. P-proximal, D-distal.

the second test (T-2A) were treated with KI and the distal ends were placed in plain agar blocks. With the third test (T-3A) the proximal and distal ends were implanted in agar blocks containing KI.

All agar blocks used in experiments performed in Groups B, and C contained 200, and 500 mg of IAA/l, respectively, in addition to the several concentrations of KI. The experiments in Group B (T-1B, T-2B, T-3B) and Group C (T-1C, T-2C, T-3C) were set up and carried out in the same manner as described for Group A with the exception of the IAA factor (Table 1).

Table 1. Sites of application of agar blocks containing the various concentrations of KI on bean leaf explants.

Test	Proximal	Distal
C - A, B. C	*	*
T-1 A, B. C	KI	*
T-2 A, B, C	*	KI
T-3 A, B, C	KI	KI

* Agar blocks without any KI.

CHAPTER IV

EXPERIMENTAL RESULTS

Presented in table 2, and figure 3 are the results of the first series of experiments (Group A) in which leaf explants were treated with KI incorporated into plain agar. After 3 days a relative high percentage of abscission was apparent among the controls of each group 92, 80, and 96%, respectively (Table 2). Tissue explants exposed to the lowest concentration of KI (1.0×10^{-4} M) showed a marked decrease in the percentage of abscission (e.g. 20, 18, 10%). This decline continued among those explants exposed to higher levels of KI. The highest concentration of iodide (5.0×10^{-4} M) almost completely inhibited the formation of the abscission zone.

The effects of KI on the abscission of the bean leaf explants when applied in blocks containing IAA at a concentration of 200 mg/l are shown in table 3, and figure 4. This level of auxin appeared to enhance the rate of abscission among the controls of each of the experiments in Group B (Table 3). On the first day of treatment 20% or more of the explants abscissed in Group B, whereas among the controls of Group A the highest percentage of abscission noticed during the first 24 hours was 16% (T-1A, Table 2). However, the total percentage of abscission among the controls of the two groups (Group A, and Group B) appeared not to differ significantly. The explants exposed to KI in Group B (Table 3, Fig. 4) followed basically the same marked inhibitory trend as noted earlier among similarly iodide treated leaf tissue in Group A (Table 2,

Table 2. Effects of KI on the rate of abscission in bean leaf explants (Group A).

KI concentration ($\times 10^{-4}$ M)	% of abscission Days after treatment 1	2	3	4	5
Group T-1A					
0.0	16	68	8	0	0
1.0	0	4	16	0	0
2.0	0	8	8	0	0
3.0	0	8	2	0	0
4.0	4	0	0	0	0
5.0	0	6	0	0	0
Group T-2A					
0.0	4	76	0	0	0
1.0	0	2	16	0	0
2.0	4	4	8	0	0
3.0	0	8	8	0	0
4.0	0	2	4	0	0
5.0	0	2	0	0	0
Group T-3A					
0.0	0	68	28	0	0
1.0	0	8	2	0	0
2.0	0	12	8	0	0
3.0	0	2	8	0	0
4.0	0	0	6	0	0
5.0	0	0	0	0	0

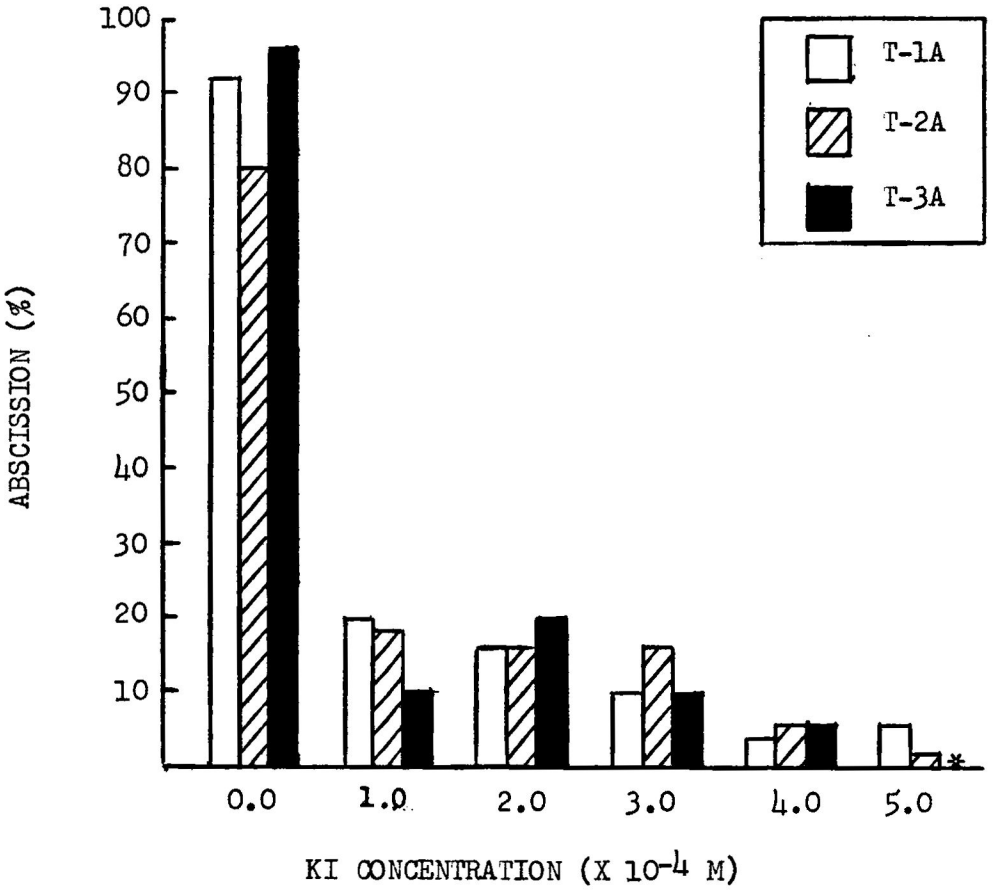

Fig. 3. Comparison of the effects of KI on abscission in bean explants when applied at different positions.

* No abscission.

Table 3. Effects of KI on the rate of abscission in bean leaf explants (Group B).

KI concentration ($\times 10^{-4}$ M)	% of abscission, Days after treatment: 1	2	3	4	5
Group T-1B					
0.0	20	76	4	0	0
1.0	0	20	0	0	0
2.0	4	16	0	0	0
3.0	4	8	0	0	0
4.0	0	12	0	0	0
5.0	0	4	0	0	0
Group T-2B					
0.0	24	68	0	0	0
1.0	4	20	0	0	0
2.0	4	16	0	0	0
3.0	8	4	0	0	0
4.0	4	0	0	0	0
5.0	0	12	0	0	0
Group T-3B					
0.0	20	54	8	0	0
1.0	0	4	0	0	0
2.0	4	8	0	0	0
3.0	0	12	0	0	0
4.0	4	4	0	0	0
5.0	0	0	0	0	0

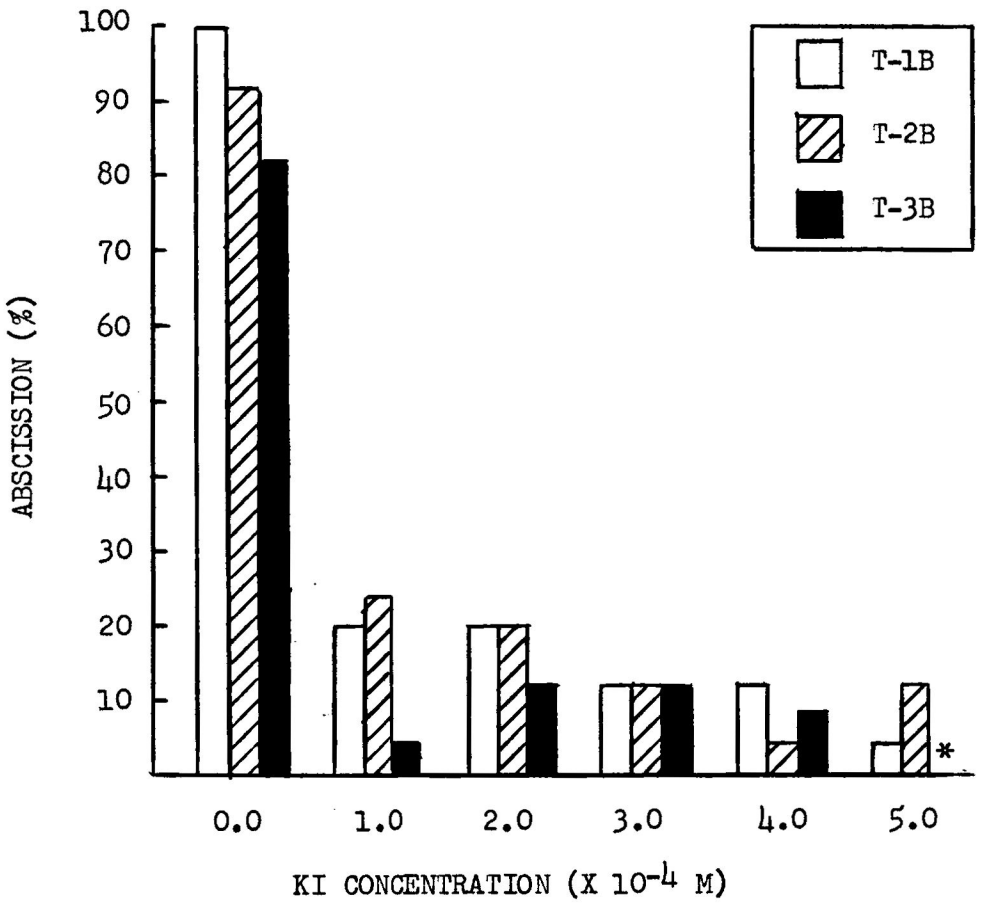

Fig. 4. Comparison of the effects of KI on abscission in bean explants when applied at different positions in a promotive level of IAA (200 mg/l).

* No abscission.

Fig. 3). The rate of abscission appeared to have been increased slightly by the addition of auxin in Group B but the total percentage seemed unaffected by the concentration of IAA.

The leaf explants in Group C (Table 4, Fig. 5) were exposed to KI in agar blocks containing IAA at a concentration of 500 mg/l. This level of auxin caused a marked retardation of abscission even among the controls (Table 4, Fig. 5), whereas the lower concentration of IAA used in Group B appeared not to materially effect abscission among any of the explants.

The level of auxin used in the experiments of Group C seemed to have greatly masked the inhibitory effect of KI (Fig. 5) which was apparent among the explants in Group A, and Group B. Throughout the lower concentrations (1.0, 2.0, 3.0, X 10^{-4} M KI) the IAA appeared to have an additive effect on the action of KI (cf. Figs. 3, 4, 5).

The application of KI at different sites (proximal, distal, proximal and distal) adjacent to the abscission zone had little, if any, effect on the rate or percentage of abscission among the different leaf explants.

The explants used in Group A (without IAA) showed marked degrees of yellowing within 2 to 3 days after treatment. This became more pronounced among explants treated with KI ranging from 3.0 to 5.0 X 10^{-4} M. The site of application of the iodide ion did not alter these results. After the yellowing occurred, in a majority of cases, the explants became very soft and showed signs of decay before the end of the observation period.

The most striking general observation among the control explants

Table 4. Effects of KI on the rate of abscission in bean leaf explants (Group C).

KI concentration ($\times 10^{-4}$ M)	% of abscission Days after treatment 1	2	3	4	5
Group T-1C					
0.0	0	12	0	0	0
1.0	4	8	0	0	0
2.0	0	0	0	0	0
3.0	4	8	0	0	0
4.0	0	12	0	0	0
5.0	0	0	0	0	0
Group T-2C					
0.0	0	8	0	0	0
1.0	0	0	0	0	0
2.0	0	12	0	0	0
3.0	0	8	0	0	0
4.0	0	0	0	0	0
5.0	0	0	0	0	0
Group T-3C					
0.0	0	8	0	0	0
1.0	0	12	0	0	0
2.0	0	8	0	0	0
3.0	0	0	0	0	0
4.0	4	4	0	0	0
5.0	0	8	0	0	0

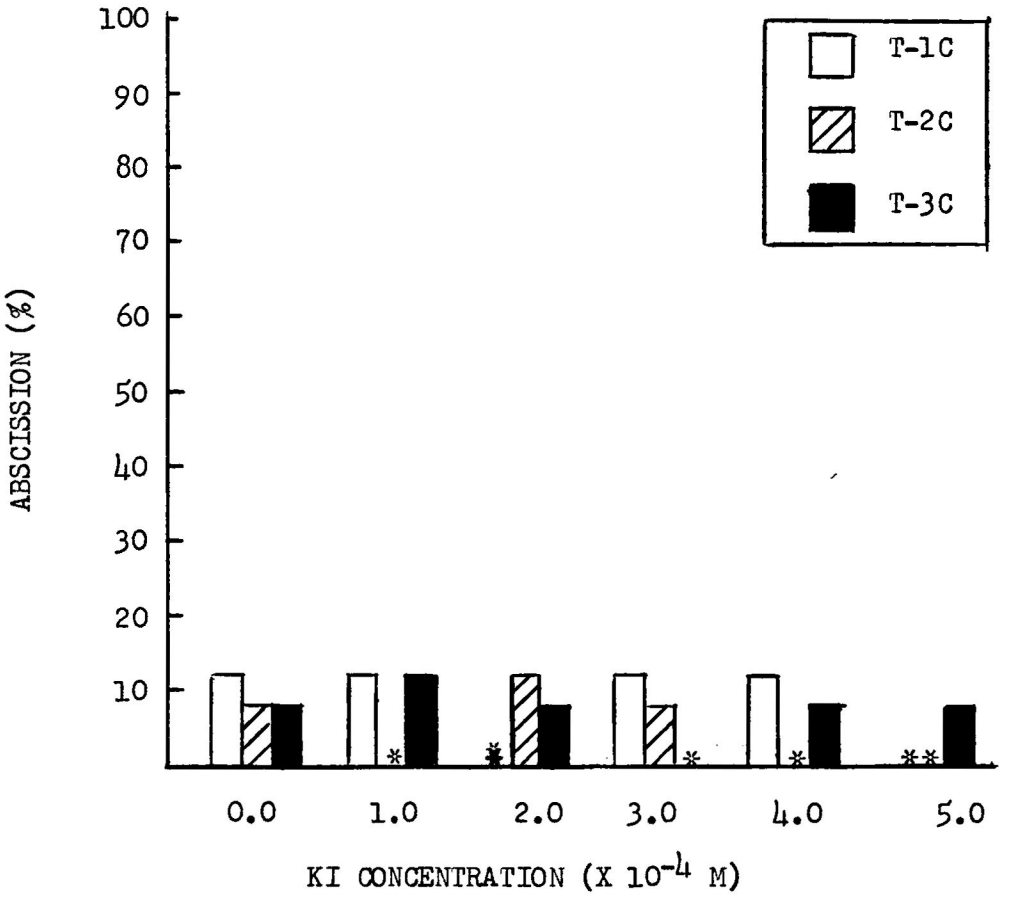

Fig. 5. Comparison of the effects of KI on abscission in bean explants when applied at different positions in an inhibitory level of IAA (500 mg/l).

* No abscission.

was that they had a tendency to remain green and vibrant during the entire 5 day observation period, even after abscission had occurred. This was evident in all controls, those treated with agar blocks containing both concentrations of IAA, and those treated with plain agar blocks.

CHAPTER V

DISCUSSION

The data obtained in this study gives no evidence that the iodide ion promotes abscission in bean leaf explants. The several treatments (KI only, KI with a promotive level of IAA (200 mg/l), KI with an inhibitory level of IAA (500 mg/l) showed little, if any, variation in total percentage of abscission is evidence for this statement. My results demonstrated that KI retarded abscission in bean leaf explants and are not in agreement with those of Boyd (6), Nolan (16), or Herrett et al. (10). They reported that the iodide ion promoted abscission in certain plants, however, several factors may have been responsible for the difference.

The first and maybe a major factor which probably contributed to the seemingly conflicting results was the specific tissues used. In this study explants from the first trifoliate leaf of bean was employed whereas Herrett and co-workers (10) treated the primary leaf (a simple leaf) of bean. They stated that prior to abscission the iodide compounds apparently caused the desiccation of the blade. It is generally known that leaf blades are major sources of distal auxin which greatly retards abscission when present in relatively high concentrations. Therefore, in the study of Herrett et al. (10) the iodide ion probably induced abscission by directly or indirectly killing the leaf blade which apparently caused a substantial reduction in the distal auxin level, thereby causing premature defoliation. They also found that highly concentrated iodide compounds induced severe desiccation which resulted in freezing the

leaves on the plants.

The concentration of the iodide ion may also have been partially responsible for the variance. The level used by Herrett et al. (10) ranged from 0.44 to 7.35 X 10^{-4} M which was higher than that employed in this study. In spite of the fact that the concentrations were higher in their work, the actual amount reaching the abscission region in the present investigation may have been considerably higher. Herrett et al. (10) cited evidence which strongly suggested to them that the iodide ion was retained within the leaf blade.

Many of the explants treated with the higher levels of KI (3.0, 4.0, 5.0 X 10^{-4} M) exhibited signs of yellowing and early decay while the controls and most of those exposed to the lower concentrations (1.0 and 2.0 X 10^{-4} M KI) remained green and vibrant during the entire 5-day test period. These results strongly suggested that higher levels of KI affected adversely the basic metabolism of the tissue or that they were somewhat toxic. Herrett et al. (10) reported that the iodide ion caused the petiole to become yellow as indicated for the rachis above.

Further evidence which demonstrated that the iodide ion retarded abscission was obtained when the KI was applied in conjunction with a low and a high level of IAA. The work of Addicott and Lynch (2) indicated that relatively low concentrations of IAA promoted abscission whereas much higher levels had the reverse effect. In this investigation the results showed that the level of IAA had no appreciable effect on the inhibitory action of the KI.

The site of application of the various concentrations of KI did not show any significant differences in the rate or total percentage of

abscission among the various bean leaf explants.

CHAPTER VI

SUMMARY

Leaf explants which included 3 mm of pulvinus tissue and 10 mm of rachis tissue were excised from the terminal leaflet of the first trifoliate leaf of bean plants. The proximal and distal ends of the explanted tissue were treated with 5 concentrations of KI (1.0, 2.0, 3.0, 4.0, 5.0 X 10^{-4} M), either incorporated in plain agar, or agar blocks containing a promotive level of IAA (200 mg/l) or agar blocks containing an inhibitive level of IAA (500 mg/l).

The data showed that the iodide ion retarded abscission substantially. This was more pronounced among explants treated with the higher concentrations of KI. The 2 levels of IAA had little, if any, influence in altering the action of the KI. Explants treated with KI at different positions (proximal, distal, proximal and distal) did not vary significantly in the rate or percentage of abscission.

LITERATURE CITED

1. Addicott, F. T. 1961. Auxin in the regulation of abscission. Encyclopedia of Plant Physiol. 14: 829-836.

2. Addicott, F. T., and R. S. Lynch. 1951. Acceleration and retardation of abscission by indoleacetic acid. Science 114: 688-689.

3. Addicott, F. T., R. S. Lynch, and R. H. Carns. 1955. Auxin gradient theory of abscission regulation. Science 121: 644-645.

4. Audus, L. J. 1963. Plant growth substances. Interscience, New York. 553 p.

5. Biggs, R. H., and A. C. Leopold. 1958. The two phase action of auxin on abscission. Amer. J. Bot. 45: 457-551.

6. Boyd, L. Q. 1955. U. S. Patent No. 2,726,149.

7. Carns, H. R. 1966. Abscission and its control. Ann. Rev. of Plant Physiol. 17: 295-314.

8. Gardner, F. E., and W. G. Cooper. 1945. Effectiveness of growth substances in delaying abscission of Coleus petioles. Bot. Gaz. 105: 80-89.

9. Gaur, B. K., and A. C. Leopold. 1955. The promotion of abscission by auxin. Plant Physiol. 30: 487-490.

10. Herrett, R. A., H. H. Hatfield, D. G. Crosby, and A. Vlitos. 1962. Leaf abscission induced by the iodide ion. Plant Physiol. 37: 358-363.

11. Jacobs, W. P. 1955. Studies on abscission: The physiological basis of the abscission-speeding effect of intact leaves. Amer. J. Bot.

42: 594-604.

12. LaRue, E. D. 1963. The effect of auxin on the abscission of petioles. Proc. Nat. Acad. Sci. 22: 254-259.

13. Leopold, A. C. 1955. Auxins and plant growth. University of California Press, Berkeley. 354 p.

14. Meyer, B. S., and D. B. Anderson 1952. Plant Physiology. D. Van Nostrand Co., New Jersey. 784 p.

15. Meyer, R. M. 1940. Effect of growth substances on the absciss layer in leaves of Coleus. Bot. Gaz. 102: 323-338.

16. Nolan, J. H. 1960. U. S. Patent No. 2,937,081.

17. Shoji, K., F. T. Addicott, and W. A. Sivets. 1951. Auxin in relation to leaf blade abscission. Plant Physiol. 26: 189-191.